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Determination of species and instars of the larvae of the Afrotropical species of *Thanatophilus* Leach, 1817 (Coleoptera, Silphidae)

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Abstract

Thanatophilus micans and *T. mutilatus* have significance for forensic entomology. Their larvae are therefore described and a key is provided for identifying the larvae of Afrotropical Silphidae based on morphological characters. It is shown that seven common species of *Thanatophilus* can be distinguished by a 360 bp mtDNA sequence from the cytochrome oxidase I gene.

Keywords

Thanatophilus, *Silpha*, Silphidae, taxonomic key, forensic entomology, Africa

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Introduction

The Afrotropical species of Silphidae, *Silpha punctulata* (Olivier, 1790), *Thanatophilus mutilatus* (Laporte*, 1840) and *T. micans* (Fabricius, 1794), can provide an estimate of the minimum amount of time for which a corpse or carcass has been dead (Midgley and Villet 2009a, Ridgeway et al. 2014). The two species of *Thanatophilus* are sufficiently different in their development and natural history that information about one cannot be used to interpret the forensic significance of the other (Ridgeway et al. 2014) and the natural history of *S. punctulata* is practically unknown (Prins 1984), so their correct identification is important to forensic entomology in Africa.

The genus *Thanatophilus* Leach, 1815 contains 24 described species distributed throughout the Holarctic and Africa (Schawaller 1981, Anderson and Peck 1985, Ratcliffe 1996). Ever-increasing international traffic allows the movement of insects between countries and even continents; for example *Silpha tristis* Illiger, 1798 has been introduced into North America from Europe (LaPlante 1997). For this reason, it is important that forensic entomology takes a less parochial view of the identification of necrophagous insects (Harvey et al. 2008) to assure the quality and fitness-for-purpose of its evidence (Amendt et al. 2015).

We therefore describe and illustrate the morphology of the larvae of *T. micans* and *T. mutilatus*, provide a key for the identification of the larvae of Afrotropical Silphidae, and show that seven common species of *Thanatophilus* can be identified by 360 bp fragments of their cytochrome oxidase I (COI) genes.

Materials and method

Larvae of *T. micans* and *T. mutilatus* were obtained from cultures originating near Grahamstown (33°19'S 26°30'E) and kept at Rhodes University. Specimens were killed and preserved in ethanol (Midgley and Villet 2009b) and voucher material was deposited at the KwaZulu-Natal Museum (*T. micans*: NMSA-COL-01339–01353; *T. mutilatus*: NMSA-COL-01354–01368). Preserved specimens of each instar of each species were examined under a Wild stereo-microscope, described, and their total length and maximum head width measured using a geometrical gauge (Villet 2007). Other specimens were cleaned in an ultrasonic bath, critical point dried and prepared for scanning electron microscopy involving a TESCAN Vega TS 5136LM scanning electron microscope. Fine morphological features were recorded from both microscopes in digital micrographs. Taxonomically relevant characters for description were chosen on the basis of previous studies (von Lengerken 1937, Dorsey 1940, Prins 1984, Anderson and

* Laporte is also cited by his title (Castelnau) rather than his surname. His full name was François Louis Nompar de Caumont Laporte, le Compte de Castelnau (Whitley 1974).

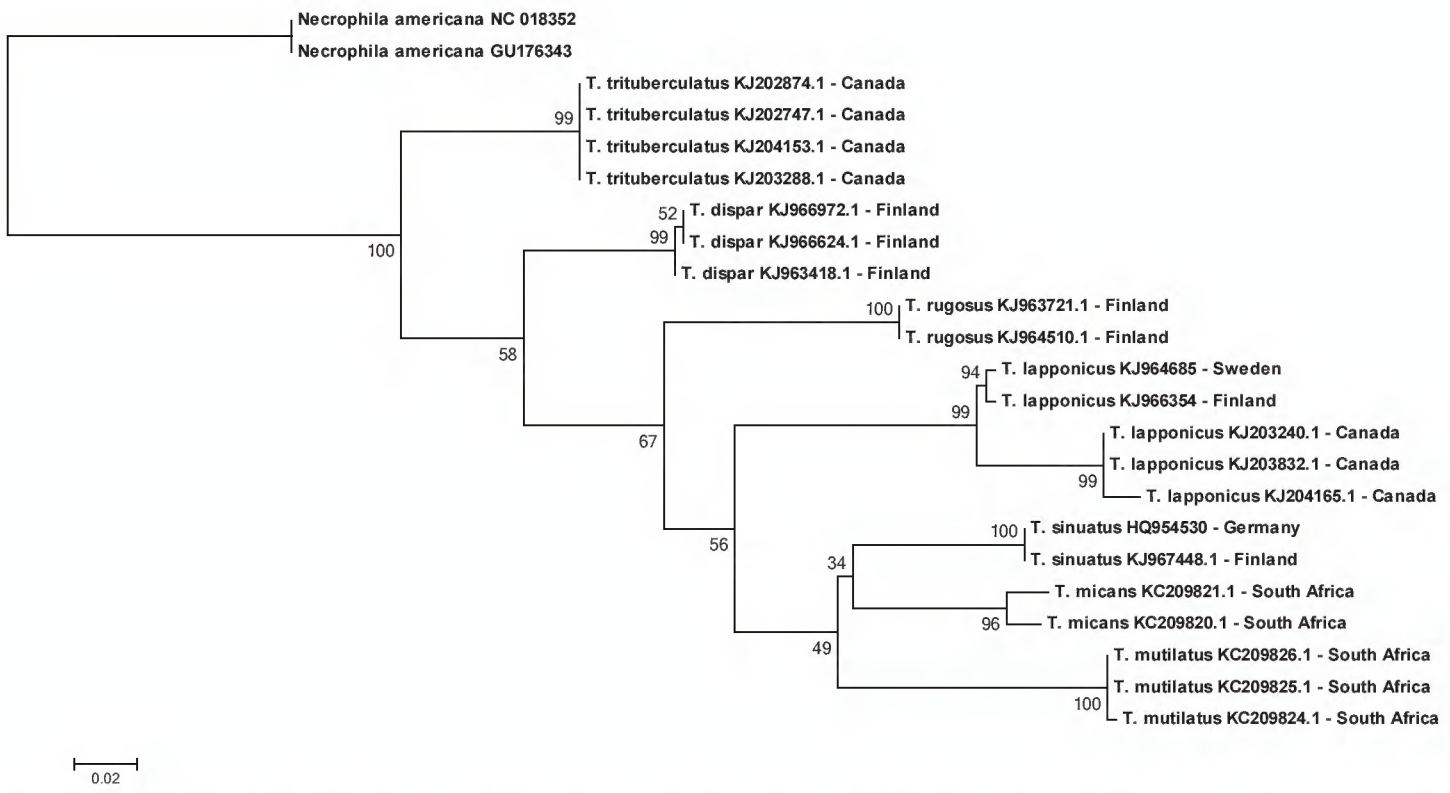

Figure 1. Maximum Likelihood phylogram with the highest log likelihood (–1507.1992) based on 360 bp of the cytochrome oxidase I gene of seven species of *Thanatophilus*. The code numbers following the specimens' identifications are their GenBank reference numbers, and the numbers beside each node are the percentage of bootstrapped trees in which the node is present. Branch lengths indicate the relative number of substitutions per site.

Peck 1985, Anderson 1987) and our own observations. Some of the character states distinguish subfamilies, while others are useful at lower taxonomic levels, e.g. genus or species. Descriptions of the larvae of *T. micans* and *T. mutilatus* were compiled from these data. A key to the Afrotropical Silphidae was compiled using our own data and Prins's (1984).

Twenty-three partial DNA sequences of the mitochondrial cytochrome oxidase I gene representing seven species of *Thanatophilus* and an outgroup (Fig. 1) were obtained from the GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and Barcode of Life (http://www.boldsystems.org/) databases. The nucleotide sequences were aligned using ClustalW in MEGA6 (Tamura et al. 2013) and trimmed to ensure that no species had missing data. A Maximum Likelihood analysis was conducted in MEGA6 based on the General Time Reversible model (Nei and Kumar 2000) with evolutionary rate differences among sites modelled with a discrete Gamma distribution (5 categories; +*G*, parameter = 0.3661) allowing for some sites to be evolutionarily invariable ([+*I*], 0.8075% of sites). A heuristic search was initiated using trees obtained automatically by applying the Maximum Parsimony method and 500 bootstrap pseudoreplicates were used to estimate the support for each node in the phylogram.

Results

Morphological identification

The morphology of the larvae of Afrotropical *Thanatophilus* is generally uniform across the three instars and between the species. Instar was most reliably determined from the maximum head capsule width of the larvae (Fig. 3).

SILPHINAE

Thanatophilus Leach, 1815

Thanatophilus mutilatus (Laporte, 1840)

Body campodeiform, approximately cylindrical, widest at metathorax, abdomen tapering from thorax; black (dark brown in teneral and preserved specimens), uniformly pigmented. Paratergites small and pointed. Dorsal intersegmental membranes with shagreened patches along anterior and posterior margins; abdominal ventral intersegmental membranes with a shagreened patch along anterior margin. Body setae with apices often notched.

Head capsule granular or tuberculate. Dorsal stemmata surrounding a small seta; ventral stemmata associated with a stout seta. First antennal segment slightly longer than third segment, with setae; third antennal segment setose with a group of minute, apical setae. Clypeus trapezoidal, granular, with three pairs of submarginal setae. Row of setae along clypeo-labral suture more distinct and with more setae than in *T. micans*. Labrum with median sclerite bearing two pairs of setae; with lateral sclerite bearing two setae. Mandible with one larger and two smaller lateroventral setae (Fig. 2). Lacinia partially fused to galea, with comb of 9–10 uniform ctenidia. Epipharynx bilobed anteriorly, with margin denticulate, without curved lateral setae. Postmentum large, with five pairs of setae.

Thoracic paraterga small, not produced into points posterolaterally, with two or more setae, particularly on posterior angle. Mesothoracic spiracle with one seta (Fig. 2). Prosternum reduced to three sclerotised areas anterior to coxae. Meso- and metasterna membraneous. Procoxa with a short groove running anteriorly.

Abdominal tergites with setae on lateral and posterior margins; paratergites weakly produced both laterally and posteriorly on segments 1–9, with posterior angles bearing a spinous seta; paratergites 1–8 projecting laterally about 25–33% of length of side. Abdominal sternites with setae on lateral and posterior margins; parasternites small, weakly developed on posterolateral angles of sternites 2–8. Abdominal sternite 5 with anterior margin more or less straight. Urogomphus 1.5 times length of abdominal segment 10, (viewed dorsally) curved inward slightly, (viewed laterally) about 1.5 times length of abdominal segment 10; basal segment about 1.5 times length of apical segment.

Larvae in each instar were most reliably separated from each other by head capsule width (Fig. 3).

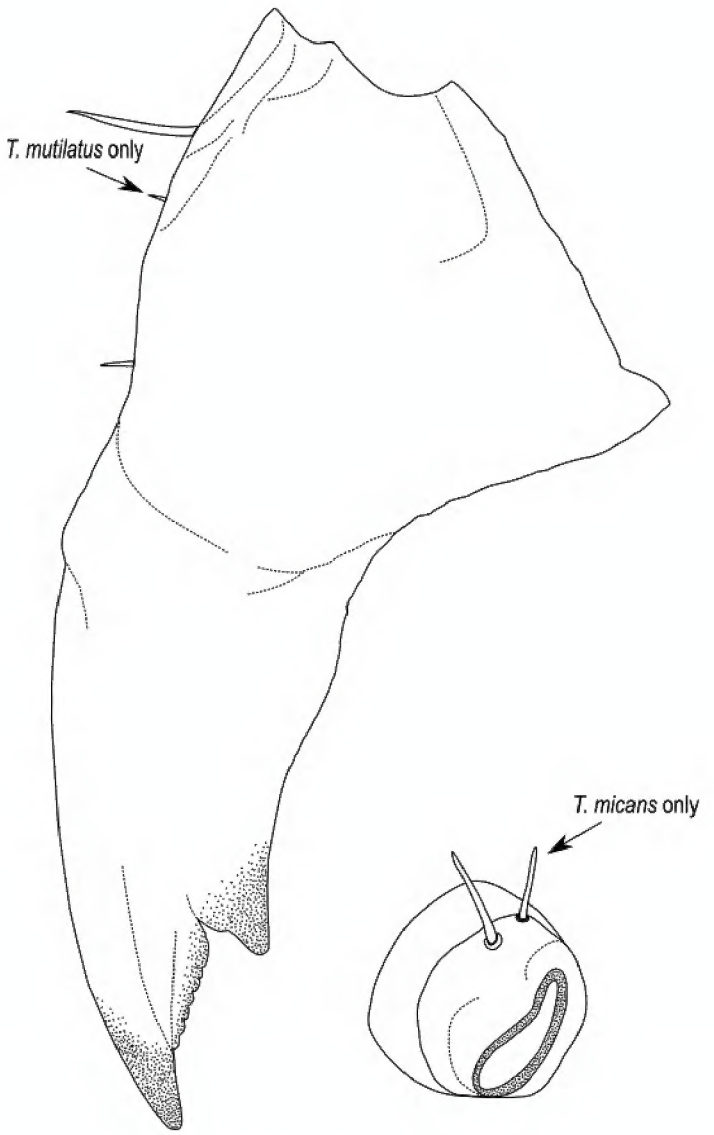

Figure 2. Right mandible in dorsal view and mesothoracic spiracle of mature Afrotropical *Thanatophilus* larvae. The arrows indicate species-specific diagnostic characteristics: *T. micans* has two ventrolateral mandibular setae and one spiracular seta, while *T. mutilatus* has three ventrolateral mandibular setae and two spiracular setae.

Thanatophilus micans (Fabricius, 1794)

Body campodeiform, somewhat dorsoventrally flattened, widest at metathorax, abdomen tapering from thorax; black (dark brown in teneral and preserved specimens), uniformly pigmented. Paratergites small and pointed. Dorsal intersegmental membranes with shagreened patches along anterior and posterior margins, abdominal ventral intersegmental membranes with a shagreened patch along anterior margin. Body setae with apices often notched.

Head capsule granular or tuberculate. Dorsal stemmata surrounding a small seta, ventral stemmata associated with a stout seta. First antennal segment about as long as third segment, without setae; third antennal segment setose with a group of minute, apical setae. Clypeus trapezoidal, granular, with three pairs of submarginal setae. Row of setae along clypeo-labral suture less distinct and with fewer setae than in *T. mutilatus*. Labrum with median sclerite bearing two pairs of setae; with lateral sclerite bearing two setae. Mandible with one larger and one smaller lateroventral seta on outer side (Fig. 2). Lacinia partially fused to galea, with comb of 9–10 uniform ctenidia.

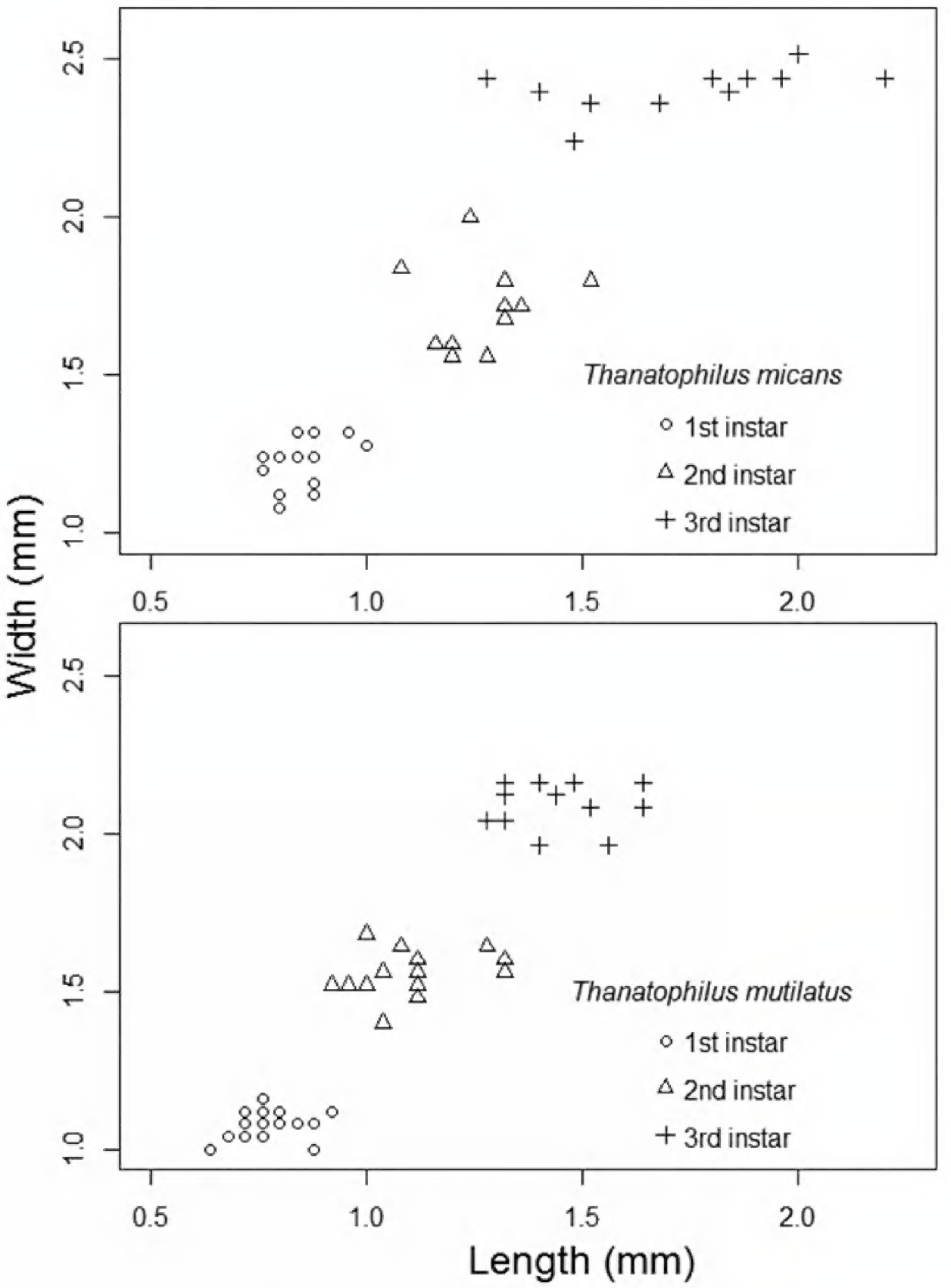

Figure 3. Scatter plot of head width against head length of larvae of *T. micans* and *T. mutilatus*. Each instar has a characteristic head width that allows individuals to be assigned unambiguously to a growth stage

Epipharynx bilobed anteriorly, with margin denticulate, without curved lateral setae. Postmentum large, with five pairs of setae.

Thoracic paraterga small, not produced into points posterolaterally, with two or more setae, particularly on posterior angle. Mesothoracic spiracle with two setae, rarely three (Fig. 2). Prosternum reduced to three sclerotised areas anterior to coxae. Meso- and metasterna membraneous. Procoxa with a short groove running anteriorly.

Abdominal tergites with setae on lateral and posterior margins; paratergites weakly produced both laterally and posteriorly on segments 1–9, with posterior angles bearing a spinous seta; paratergites 1–8 projecting laterally about 25–33% of length of side. Abdominal sternites with setae on lateral and posterior margins; parasternites small, weakly developed on posterolateral angles of sternites 2–8. Abdominal sternite 5 distinctly convex. Urogomphus 2 times length of abdominal segment 10, (viewed dorsally) straight, (viewed laterally) about two to three times length of abdominal segment 10; basal segment about two or three times length of apical segment.

Larvae in each instar were most reliably separated from each other by head capsule width (Fig. 3).

Comparisons

Thanatophilus micans has longer urogomphi than *T. mutilatus* in all three instars, and one seta on the ventral edge of the mandible, rather than two (Fig. 2). It differs from *T. mutilatus* and *S. punctulata* by having two setae (occasionally three) on the mesothoracic spiracle (*T. mutilatus* has one and *S. punctulata* has none) (Fig. 2). While *T. micans* is generally larger in all larval stages than *T. mutilatus* (Fig. 3), there is still overlap. Species are most reliably determined by mesothoracic setae, while instar within species can be reliably determined by maximum head capsule width.

Key to larvae of Afrotropical Silphidae

1 Body widest at metathorax; abdomen about 2.0 times length of thorax; mesothoracic spiracle without setae..... ***Silpha punctulata***

– Body widest at prothorax; abdomen about 2.5 times length of thorax; mesothoracic spiracle with setae..... **2**

2 Mesothoracic spiracle with one seta, abdominal sternite 5 with anterior margin more or less straight; urogomphus curved inwards slightly, 1.5 times length of abdominal segment 10..... ***Thanatophilus mutilatus***

– Mesothoracic spiracle with two setae; abdominal sternite 5 distinctly convex; urogomphus straight, 2.0–3.0 times length of abdominal segment 10 ..... ***Thanatophilus micans***

Molecular identification

There was a total of 360 bp in the trimmed partial COI sequences. These allowed the specimens to be placed with conspecifics in the phylogram with bootstrap support of 96–100% (Fig. 1). Support for the relationships amongst the species was generally very poor.

Discussion

Taxonomy

Previous descriptions of the 3rd-instar larvae of *T. micans* and *S. punctulata* recorded four setae present on either side of the labrum; one mandibular seta; and two pairs of setae on the distal region of the postmentum (Prins 1984). This study observed five labral setae; two mandibular setae; and a third pair of smaller setae at the extreme proximal region of the postmentum. Prins (1984) also noted two setae on the mesothoracic spiracle of *T. micans* and none in *S. punctulata*. Whether this represents geographical variation is unclear because Prins' material could not be found at his former institution and no other western *T. micans* larvae were available.

The number of mesothoracic spiracular setae is a character that can be used to differentiate the mature larvae of all three species of Silphidae in South Africa. It has no overlap between the species and is easily measured with a stereomicroscope. The number of setae on the mandible can be used to determine the larvae of African *Thanatophilus*, but electron microscopy is required to make this measure repeatable. Using light microscopy does not guarantee that all setae will be visible, a possible explanation for Prins' (1984) description of *T. micans* mentioning only one mandibular seta.

Phylogenetic analysis of preliminary molecular data indicated that *Thanatophilus* is a well-supported monophyletic genus (Dobler and Müller 2000). The relatively poor support found here (Fig. 1) for the relationships between seven species of *Thanatophilus* was also found using 2094-bp of mitochondrial DNA from four species (Dobler and Müller 2000), so it is not entirely due to the brevity of the sequences used here. The very strong support for placing conspecific individuals together (Fig. 1) suggests that short DNA sequences could identify common species of *Thanatophilus*.

To differentiate the instars of silphid larvae, previous authors have estimated body size using the minimum distance between the dorsal stemmata, and/or the maximum widths of the pronotum and mesonotum (Watson and Carlton 2005, Velásquez and Viloria 2010, Frątczak and Matuszewski 2016). Because of extreme collinearity between the two thoracic width measurements, Frątczak and Matuszewski (2016) used only pronotal width and interstemmatal distance in their multivariate classification analyses of four European species of Silphidae, and found that the distance between the stemmata was the most influential estimator in their DFA. Similarly, we found head width to be more diagnostic that body length (Fig. 3), probably because the body shape of *Thanatophilus* larvae changes, both within each instar are they grow, and at ecdysis, when the same body volume becomes accommodated in a larger exoskeleton. Frątczak and Matuszewski (2016) also found categorical variation in size between instars of four European silphids. It is likely that, being more rigid than the other tagmata, the head will be the most reliable single estimate of instar (whether measured as maximum width or as interstemmatal distance).

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